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# **Inhibitory effect against reverse transcriptase of HIV-1 of the extract of selected medicinal plants**

**Swapna K**

Department of Zoology, Kakatiya University, Warangal-506009, TS, India  
Corresponding author Email: [swapna.kandagatla2016@gmail.com](mailto:swapna.kandagatla2016@gmail.com)

**Srujana M**

Chaitanya Deemed to be University, Department of Pharmacy, Kishanpura, Hanamkonda, Warangal – 506001 TS, India

**Estari M**

Department of Zoology, Kakatiya University, Warangal-506009, TS, India  
Corresponding author Email: [drestari@kakatiya.ac.in](mailto:drestari@kakatiya.ac.in)

**Abstract**--HIV is a virus that multiplies inside of your body and impairs your defences. This makes it more difficult to fend against common illnesses and bacteria. The study's goals included preparing a crude extract from specific medicinal herbs and determining its ability to inhibit HIV-I reverse transcriptase in vitro. This study aimed to conduct an ethnobotanical survey of the healing plants used by Telangana state's indigenous people. Five distinct medicinal plant parts were chosen from a variety of plants, and the % yield was measured after sequential maceration with the five different solvents n-hexane, chloroform, ethyl acetate, acetone, and methanol. Using the RetroSys kit, the HIV reverse transcriptase enzyme inhibition was carried out on crude extracts. *Withania somnifera* demonstrated excellent inhibitory activity with a range of 40.5 percent to 72.5 percent from a concentration of 0.62 mg/ml to 10 mg/ml, which is close to nevirapine, the control, at 75.3 percent and an effective IC<sub>50</sub> value of 1.40 mg/ml used as a lead molecule. HIV-RT inhibition for the aforementioned plants was determined with a range of 32.5 to 72.8 percent. The target molecule can be found by doing research to isolate and characterize the chemical ingredient that makes *Withania* methanol extract active.

**Keywords**--HIV-1, *Andrographis*, *Ocimum*, *Calotropis*, Reverse transcriptase.

## **Introduction**

The HIV virus, one of the most serious public health problems in the world, is what causes AIDS. HIV infection is the root cause of the immunodeficiency virus. Our immune system's capacity to fight infection is compromised when the virus affects CD4 cells and kills them. Reduced immunity (AIDS) is the cause of acquired immunodeficiency virus (Alvi et al, 2018). There are 38 million HIV-positive people in the world. Around 1.8 million kids under the age of 14 have HIV. The number of new infections increased by 72% in Eastern Europe and Central Asia (Alvi et al, 2018). In 2020, there will be about 37.6 million people living with HIV worldwide, according to UNAIDS. There were 1.7 million kids under the age of 15 and 35.9 million adults. There will have been an estimated 1.5 million new HIV infections by the year 2020. Antiretroviral therapy (ART) is already available to manage HIV infection, but due to ART's limitations, researchers throughout the globe are investigating and creating more dependable and secure drugs derived from natural sources to treat HIV infection (Estari et al, 2012).

Natural substances made from plants have biological effects, such as an anti-HIV effect (UNAIDS, 2016). Based on folkloric medicine, a variety of native medicinal plants have been chosen and looked into for its potential antiretroviral effects. According to several researches, substances derived from plants have anti-HIV-1 effects that target different stages of HIV replication. Reverse transcriptase (RT) assays were used in this study to assess the HIV-1 inhibitory activity of five different solvent extracts of Indian medicinal plants: *Andrographis paniculata* leaves (Fabaceae), *Withania somnifera* stem (Fabaceae), *Calotropis procera* roots (Asclepiadoideae), *Phyllanthus niruri* fruit (Phyllanthaceae), and *Ocimum sanctum* leaves (Labiatae). All of the herbs are native to India and have been utilised for a long time in both primary and combined treatments of ailments recommended by conventional folk medicines (Sanhit et al, 2021). The purpose of the study was to synthesize a crude extract from various medicinal plants and to look into the in vitro activity against HIV-I reverse transcriptase.

## **Materials and Methods**

### **Study Area**

In the tribal districts of Telangana's Adilabad, Warangal, and Khammam, an ethnobotanical survey was conducted (Figure-1). The local populace as well as the native Koya and Kolam tribes were the subjects of the ethnobotanical survey. We met the tribal community where they lived. The research region was visited on the field multiple times (during the study period).

Through questionnaires, interviews, and conversations with tribal practitioners, ethnobotanical data were gathered. The questionnaire allowed for a descriptive response regarding the prescribed plant, including the part of the plant used, medicinal uses, and specific information about the mode of preparation (e.g., decoction, paste, powder, and juice), form of usage (fresh or dried), and mixtures of other plants used as ingredients.

### **Collection of Plant Material**

The chosen plants were gathered from the Telangana State locations mentioned above, and they were then validated by a taxonomist from the department of botany's plant systematic lab at Kakatiya University in Warangal.

### **Preparation of Crude Extracts**

The gathered plant materials were air dried in the shade and ground into a fine powder using a homogenizer. The 100 grams of powdered material were extracted using the sequential maceration method with solvents that increased in polarity, including hexane, chloroform, ethyl acetate, acetone, and methanol for each solvent. Using a rotating evaporator and lowered pressure, the extracts were concentrated to a constant weight. The extracts were gathered and stored in a desiccator until they were needed for additional research.

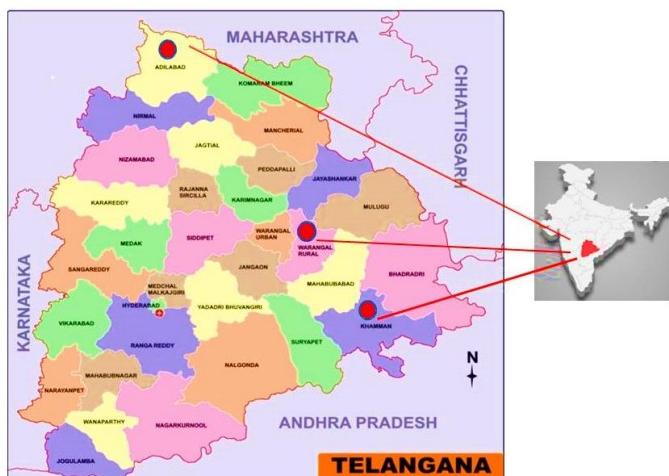


Figure-1. Study area for the ethno botanical survey of the medicinal plants used by tribals of Telangana state (Adilabad, Warangal and Khammam, in red colour spots) India

The residual extract's weight was determined, and the following percent yield calculation was made: Extract yield percent is equal to  $W_1/W_2 \times 100$ , where  $W_1$  is the net weight of the extracted powder in grams.  $W_2$  is the total weight in grams of the powder used for extraction. The weights of the five distinct solvent extracts from *Andrographis paniculata* are 0.345g, 0.461g, 0.474g, 0.391g, and 2.02 g. (hexane, chloroform, ethyl acetate, acetone and methanol respectively). Among all the solvent extracts, the methanol crude extract had the highest yield (Figure-2).

### **HIV Reverse Transcriptase Activity**

With the afore mentioned crude extracts (methanol crude extracts of *Andrographis paniculata*, *Withania somnifera*, *Phyllanthus niruri*, *Ocimum sanctum*, and ethyl acetate crude extract of *Calotropis procera*), HIV-RT reverse

transcriptase (RT) activity, and the RetroSys kit, the HIV reverse transcriptase enzyme inhibition was carried out (Innovagen, Lund, Sweden). With a final DMSO content of 0.5 percent v/v, all of the crude extracts were initially examined at a concentration of 100 mg/mL. 20 µL of HIV1 RT enzyme (0.2 ng/mL) and 20 µL of a reaction mixture containing biotin and digoxigenin-labeled deoxyuridine triphosphate were combined to perform the experiment (biotin-dUTP and DIG-dUTP). The mixture was heated to 37°C for an hour, transferred to the wells of microplate modules, and then heated there for an additional hour. Complete removal of the solution was followed by five 30-second well rinses with washing buffer. After that, 200 µL of an anti-DIG-POD antibody was added to the wells of the microplate module, and it was incubated there for an hour at 37°C. Microplate wells were then washed once more. The microplate wells were then filled with 200 µL of the ABTS substrate solution, which was then incubated for around 30 minutes. The amount of green colour development was deemed sufficient, and a microplate reader was used to test the sample's absorbance at 405 nm. Negative control was a reverse transcriptase inhibitor called nevirapine (1 mg/mL).

## **Results and Discussions**

### ***Ethnobotanical Survey***

In the research region, there were 8 informants ranging in age from 35 to 70. Two of them were farmers, and six of them regularly used herbs. We were joined by them as they took us to a wooded area and showed us some of the plants they use to make their traditional remedies.

With the assistance of specialists from Kakatiya University, Warangal, and the Department of Botany, the collected plant specimens were accurately identified. Following proper processing, the specimens were eventually placed in the herbariums of my department, Kakatiya University, and Warangal. They were described in terms of their applications, therapeutic significance, method of administration, and regional names. Information is tabulated with the plant name, family, local name, parts utilised, preparation technique, and utility.

To examine the in vitro anti-HIV activity, a total of 5 plants were chosen based on the information provided by local tribal healers in the tribal areas of Adilabad, Warangal, and Khammam in the Telangana district. From the aforementioned locations, the following five plants were gathered:

- |  |           |
|--|-----------|
| 1. <i>Androgaphis paniculata</i> (king of bitters-Neelavemu) | Leaves    |
| 2. <i>Withania sominifera</i> (Indian ginseng-Ashwagandha)   | Stem bark |
| 3. <i>Phyllanthusniruri</i> (stonebreaker-Nelausiri)         | Fruits    |
| 4. <i>Ocimum sanctum</i> (holy basil-Tulasi)                 | Leaves    |
| 5. <i>Calotropis procera</i> (apple of Sodom -Arka)          | Roots     |

### **Yield of crude extracts**

Table-1 shows the percent yield of extracts obtained from different parts of five plants. The yields expressed are based on the dried weight of sample raw materials. The weights of the five distinct solvent extracts from *Andrographis paniculata* are 0.345g, 0.461g, 0.474g, 0.391g, and 2.02g (hexane, chloroform, ethyl acetate, acetone and methanol respectively). Comparing all the solvent extracts, the methanol crude extract had the highest yield. There are five distinct solvent extracts from *Withania somnifera*, each weighing 0.306 g, 0.244 g, 0.146 g, 0.134 g, and 2.496 g. (hexane, chloroform, ethyl acetate, acetone and methanol respectively). Among all the solvent extracts, the methanol crude extract had the highest yield (2.5 g).

The weights of the five distinct solvent extracts from *Phyllanthus niruri* are 0.523 g, 0.396 g, 0.191 g, 0.093 g, and 2.476 g. (hexane, chloroform, ethyl acetate, acetone and methanol respectively). Among all the solvent extracts, the methanol crude extract had the highest yield (2.476 g).

Table-1: Percent yield of extract from selected five plants

Sl. No	Name of a plant	Part of a plant taken	Solvent used	Weight obtained	Extract yield in percent
1	<i>Andrographis paniculata</i>	Leaves	n-hexane	0.345g	0.345
			Chloroform	0.461g	0.461
			Ethylacetate	0.474g	0.474
			Acetone	0.391g	0.391
			Methanol	2.02g	2.02
2	<i>Withania somnifera</i>	Stembark	n-hexane	0.306g	0.306
			Chloroform	0.244g	0.244
			Ethylacetate	0.146g	0.146
			Acetone	0.134g	0.136
			Methanol	2.496g	2.496
3	<i>Phyllanthus niruri</i>	Fruit	n-hexane	0.523g	0.523
			Chloroform	0.396g	0.396
			Ethylacetate	0.191g	0.191
			Acetone	0.093g	0.093
			Methanol	2.476g	2.476
4	<i>Ocimum sanctum</i>	Leaves	n-hexane	0.264g	0.264
			Chloroform	0.514g	0.514
			Ethylacetate	0.119g	0.119
			Acetone	0.182g	0.182
			Methanol	1.012g	1.012

5	<i>Calotropis procera</i>	Roots	n-hexane	0.453g	0.453
			Chloroform	0.877g	0.877
			Ethylacetate	1.008g	1.008
			Acetone	0.374g	0.374
			Methanol	0.585g	0.585

There are five distinct solvent extracts from *Ocimum sanctum*, weighing 0.264g, 0.514g, 0.119g, 0.182g, and 1.012g (hexane, chloroform, ethyl acetate, acetone and methanol respectively). Among all the solvent extracts, the methanol crude extract had the highest yield (1.01 g).

*Calotropis procera* roots were extracted using five different solvents at weights of 0.453g, 0.877g, 1.008g, 0.374g, and 0.585g (hexane, chloroform, ethyl acetate, acetone and methanol respectively). Comparing all of the solvent extracts, the ethyl acetate crude extract had the highest yield (1.008 g).

Various plant elements, such as the chemical makeup of the plant, the makeup of the soil, and agro-climatic conditions, may induce variations in the percent yield of extracts. The ability of the extraction solvent to dissolve endogenous chemicals may be one of the additional variables. Other than the ethyl acetate extract of the root of *Calotropis procera*, all other plant extracts in methanol generated the highest yield (Figure-2).

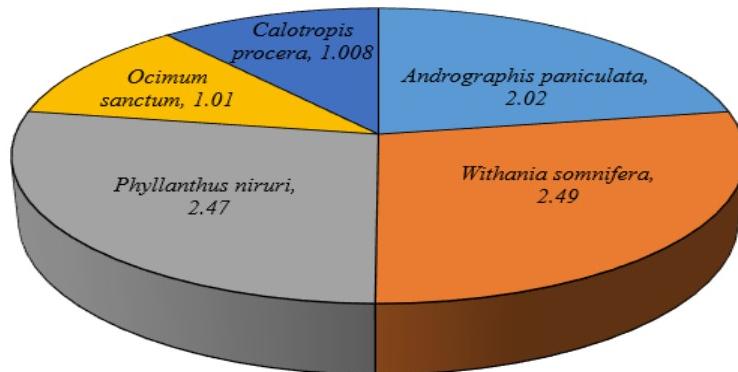


Figure-2.Percent yield obtained highest in different plants

The mean and standard deviation (mean SD) of the data were displayed. Values are average of 5 replications plus SEM. The formula shown below was used to compute the % inhibition. percent preventing HIV-1 RT enzyme = 100 times the control divided by the sample Where; Absorption intensity of control equals A control Plant crude extracts with better than 50% inhibition activity were chosen as the A sample (A sample = Absorption intensity of sample) for the 50 percent inhibitory concentration (IC50). The following five strengths of plant crude extracts were prepared: 0.62, 1.25, 2.5, 5 and 10 mg/mL. The above-described assay procedure was followed. The logarithmic equation produced by Microsoft Excel 2013 from five distinct concentrations was used to calculate the IC50 value

from the five percentage values of the concentrations calculated from the aforementioned equation.

### HIV-1 RT activity

Five distinct crude extracts from five different plant species were tested for anti-HIV-1 reverse transcriptase (anti-HIV-1 RT). Table-2 and Figures 3–7 display the findings. Table-2 displays the plant crude extracts 50% inhibitory concentration ( $IC_{50}$ ) for HIV-1 reverse transcriptase. All of the plant's raw extracts had HIV-1 RT inhibition rates of at least 50%. Among the investigated plant crude extracts, *Withania somnifera*'s methanol extract appeared to have considerably stronger anti-HIV-1 RT activity ( $PI=72.8\pm3.8$  percent and  $IC_{50}=1.40$  mg/ml).

Table-2. Percentage of HIV-1 RT inhibition at different concentration

Sl.No	Name of the Plant	Crude Extract	Percentage of HIV-1 RT inhibition at different concentrations					$IC_{50}$ values
			0.62 mg/mL	1.25 mg/mL	2.5 mg/mL	5 mg/mL	10 mg/mL	
1	<i>Andrographis paniculata</i>	Methanol	32.5±2.8	38.3±2.4	47.5±3.2	55.4±3.2	64.4±4.2	4.62
2	<i>Withania somnifera</i>	Methanol	40.5±2.9	49.2±2.2	58.5±3.8	67.3±4.3	72.8±3.8	1.40
3	<i>Phyllanthus niruri</i>	Methanol	28.4±1.2	36.4±2.8	41.4±2.4	48.5±3.3	51.5±4.3	7.94
4	<i>Ocimum sanctum</i>	Methanol	17.4±1.1	28.5±2.3	35.2±2.6	48.8±3.2	54.6±3.5	7.50
5	<i>Calotropis procera</i>	Ethyl acetate	33.5±2.7	48.4±2.4	55.3±2.9	61.6±2.2	68.3±3.2	2.74

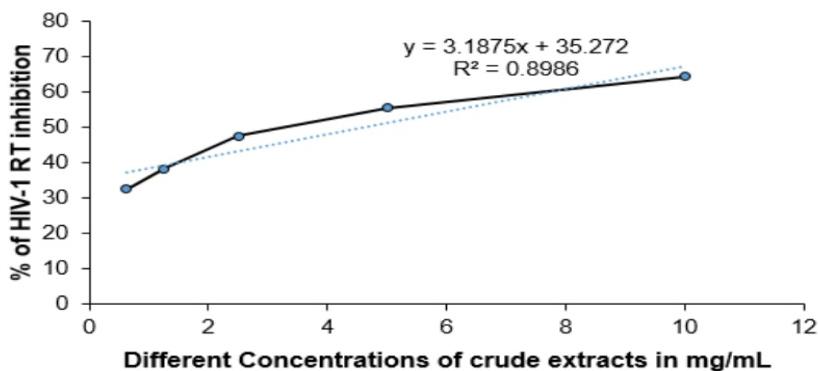


Figure-3. HIV-1 reverse transcriptase inhibition activities of *Andrographis paniculata* Methanol crude extracts

*Phyllanthus niruri*, *Ocimum sanctum*, and *Calotropis procera* ethyl acetate crude extracts all inhibited this enzyme in the ranges of 28.4 to 51.5 percent with  $IC_{50}$  of 7.94 mg/ml, 17.4 to 54.6 percent with  $IC_{50}$  of 7.50 mg/ml, and 33.5 to 68.3

percent, respectively. *Andrographis paniculata* methanol crude extract showed inhibition in the range (Table-2).

While methanol extracts of *Phyllanthus niruri* and *Ocimum sanctum* showed less HIV-1 RT inhibition, ethyl acetate crude extracts of *Calotropis procera* and *Andrographis paniculata* showed considerable HIV-1 RT inhibition (Figure-3-7). The maximum level of action for all plant crude extracts is 10 mg/mL at higher concentrations. Nevirapine demonstrated 75.3 percent HIV-1 RT suppression, which is comparable to the *withania somnifera* plant's methanol crude extract.

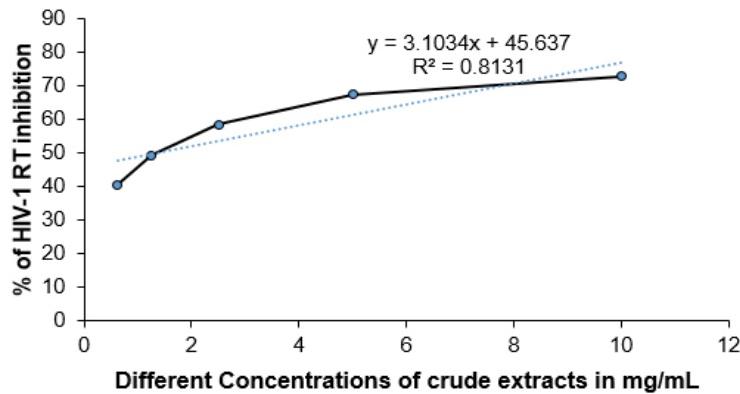


Figure-4. HIV-1 reverse transcriptase inhibition activities of *Withania somnifera* methanol crude extracts

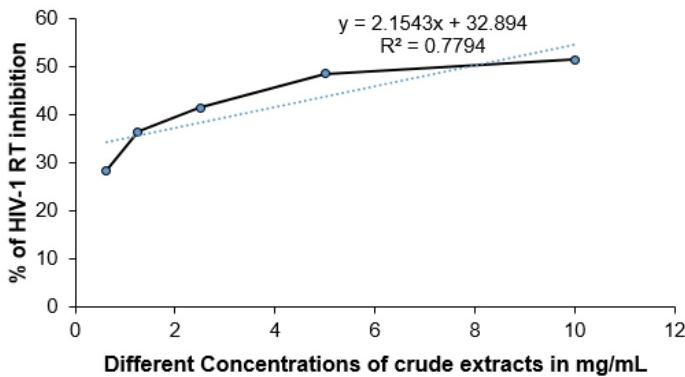


Figure-5. HIV-1 reverse transcriptase inhibition activities of *Phyllanthus niruri* Methanol crude extracts

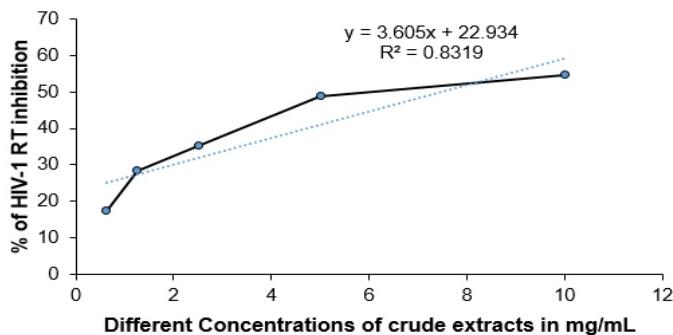


Figure-6. HIV-1 reverse transcriptase inhibition activities of *Ocimum sanctum* Methanol crude extracts

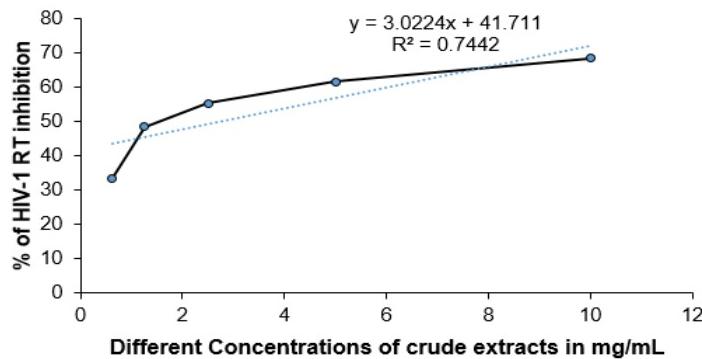


Figure-7. HIV-1 reverse transcriptase inhibition activities of *Calotropis procera* Ethyl acetate crude extracts

*Withania somnifera* methanol crude extracts demonstrated lower IC<sub>50</sub> values (1.40 mg/mL), indicating strong lead molecule inhibition of HIV-1 RT (Figure-4).

## Discussion

From 23 plants, 92 extracts were created. An HIV-1NL4.3-infected human CD4+ T-cell line called CEM-GFP cells underwent anti-HIV activity testing. In CEM-GFP cells infected with HIV-1NL4.3, viral production was considerably decreased by nine extracts from eight different plants (Sudeep Sabde et al, 2011). In another article, it is revealed that the SensoLyte® 520 HIV-1 protease assay fluorimetric kit and the HiLyte FluorTM488/QXLTM520 fluorescence resonance energy transfer (FRET) peptide were used to determine the inhibitory effect of a water extract of *C. edulis* leaves against HIV-1 protease activity (Beauty E et al, 2020). Neochlorogenic acid, a substance with anti-HIV activity that was found in this study by screening Cortex Mori (Jing Lia et al, 2021). For the treatment of seven different illnesses and the symptoms of HIV disease, 43 plant species from 31 families were found in our previous survey. The bulk of plant parts were ranked

beginning with the leaves (Swapna Gurrapu *et al*, 2016 and Swapna Gurrapu et al, 2013).

Andrographolide was isolated from *Andrographis paniculata* and tested for its capacity to inhibit HIV-1 reverse transcriptase using column chromatography and high performance liquid chromatography (HPLC). The MTT assay was used to test andrographolide's capacity to maintain cell viability (Swapna Gurrapu *et al*, 2017). The ability of methanolic-aqueous extracts from 70 different plants to inhibit HIV-1 reverse transcriptase activity *in vitro* was investigated. Two extracts completely inhibited the enzyme, while four extracts showed better than 90% inhibition (Rajendra Prasad Gujeti *et al*, 2014). *Geranium phaeum* has an IC<sub>50</sub> of 0.067 mg/ml, while *sambucus racemosa* has an IC<sub>50</sub> of 0.017 mg/ml (Mlinarie A *et al* 2000). In the study, *tinospora cordifolia* leaf extract exhibits 60% HIV RT suppression activity at a concentration of 20 g/ml (Estari M *et al*, 2011).

## **Conclusion**

The study's conclusions led us to select a number of therapeutic plants from tribal areas. Five different solvents were used to calculate the % yield of five different medicinal plants: n-hexane, chloroform, ethyl acetate, acetone, and methanol. Utilizing methanol, *Withania somnifera* achieved a maximum yield of 2.49g. Then, HIV-RT inhibition levels for the aforementioned plants were assessed; they ranged from 32.5 to 72.8 percent. Additionally, *withania somnifera*, which is similar to nevirapine, exhibited a maximal inhibition of 72.8 percent at 10 mg/ml. The IC<sub>50</sub> with the highest potency is 1.40 mg/ml. The target molecule can be found by doing research to isolate and characterize the chemical ingredient that makes *withania* methanol extract active.

## **Acknowledgments**

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